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ON

THE MICROSOMAL RIBONUCLEOPROTEIN

PARTICLES.

RESPONSIBLE INVESTIGATOR

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PHYSICO-CHEMICAL STUDIES
ON
THE MICROSOMAL RIBONUCLEOPROTEIN PARTICLES

The First Quarterly Progress
Report on the Second Year

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I. THE SCHEME OF THE INVESTIGATION IN THE SECOND YEAR.

1. In the last year, we studied chiefly the physicochemical properties of liver ribosome and its RNA. Recently, however, a new physical property of the nucleic acid, a ferromagnetic one, was described by Blumenfeld(1). Using an electromagnet in our laboratory, therefore, studies on such a somewhat unusual property will be made.

2. The liver microsomes were chiefly studied by many investigators hitherto, but little attention was paid to those of brain. In contrast to ribosome, the term, "microsome" can not be, strictly speaking, uniquly defined from the biochemical point of view. In view of the importance of microsomal particles in the brain function, we will study the isolation method of brain microsomal fraction and their biochemical characteristics.

II. RESULTS OBTAINED TO DATE

1. Magnetochemical property of the ribosome and the nucleic acid.

It has been already reported (2,4,5) that DNA showed a broad electron spin resonance (ESR) spectra similar to that of ferromagnetic substance. In our laboratory, it was also observed in the experiments on the effect of γ-irradiation on the free radical formation that such a broad ESR spectra of RNA and DNA appear which we attributed to the background noise at that time (3). Hence we studied the magnetic susceptibility (χ) and ESR spectra of the ribosomes, RNA and DNA.

The ribosomes and its RNA were prepared as described in the previous report, while DNA was prepared from calf thymus gland by the conventional Hammersten's method.

The ferromagnetic behavior of these substances could be confirmed by their χ measurement as well as by ESR spectra. To exclude the contribution of iron as a ferromagnetic impurity, samples were dialysed for several hours in the presence of EDTA. Only the trace or none of iron was found in the samples of nucleic acids. In the ribosomes, however, considerable amount of iron could be detected, which was hardly eliminated with a mild procedure. Hence further experiments on the ribosome were abandoned.

Such a ferromagnetism of nucleic acids was highest at pH of about 8 and disappeared in the alkaline media (pH above 10). After hydrolysis of the acids, they became diamagnetic and their ferromagnetic behavior could not be recovered by neutrolisation of hydrolysates.

The isolated nuclei as well as the DNA extracted from them was found also ferromagnetic, but the protein prepared from them never.

From the experimental results mentioned above, it seems highly probable that RNA and DNA of high molecular weight are ferromagnetic in themselves, and hence they would be so even in the ribosomes. If their magnetic property results from the trace of iron, it should originate from a specific structure of iron stabilized on the nucleic acid molecules, for instance colloidal micells of hydroxide, whose breakdown by alkali causes disappearance of observed magnetic behavior.

2. Effect of magnetic field on the ribosomes.

If the magnetic properties of RNA has a physiological significance in the role of ribosomes as a field of protein biosynthesis,

strong magnetic field would affect the ribosomal activity of incorporating amino acids. Using liver ribosomes as well as those prepared from the reticulocytes of rabbits made anemic by phenyl-hydrazine administration, incorporation rate of C^{14}-leucine was studied. The field strength of 2×10^4 gauss depress the rate by about 15-20 %, while it showed hardly any effect on the oxygen uptake of liver slice, and blood cell suspension. But further experiments would be required to draw any conclusion on the nature of such an effect.

III. RESEARCH PLAN AT THE NEXT QUARTER

1. Acomplishment of the experiments made in the first quarter period.

   Some observations stated above will be repeated.

2. Studies on the isolation of brain microsomes and ribosomes.

   Using the ultracentrifugation in the density gradient, isolation of microsomes and ribosomes will be attempted on the rabbit brain. If possible, a systematic examination of fractionation method of subcellular particles will be made.

3. Electron-microscopical and biochemical studies on the brain microsomes.

   On the microsomal fraction thus isolated, its morphological and biochemical studies will be made.

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